MATHEMATICAL ANALYSIS OF ASYMMETRIC

BIORHYTHM WAVE FORMS

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ABSTRACT

A method for analyzing asymmetric biorhythm wave forms is presented utilizing a curve fitting technique based on the method of moments and solutions of the Volterra Integro-Differential Equation. The mathematical derivation is given as well as a worked example using heart-rate (HR) data collected from a male chicken. The asymmetric HR wave form could be separated into three component curves, an ascending logistic, a descending logistic and a unimodal curve (similar in form to a skewed normal distribution), the parameters of which describe heart-rate changes and the trajectory of the wave form. While these parameters cannot as yet be related to specific physiological mechanisms, the results suggest hypotheses which may be tested experimentally. The implications of the mathematical model in the understanding of biorhythms are discussed.

INTRODUCTION

A major activity of this laboratory has been the continuous monitoring of heart rates (HR), deep body temperatures (DBT), and locomotor activity (LA) of chickens and primates, utilizing biotelemetry. Fryer et al. (1966) have described the telemetry system. Data are collected at 6-minute intervals and are reduced and analyzed, utilizing a computer program whose major features have been described by Winget et al. (1968). The mathematical basis of this computer program is that of harmonic analysis, the technique by which biological time series data have been classically analyzed.

In general, harmonic analysis provides estimates of the periodicity of the data as well as of amplitudes and phases of the periods. Biologists have been particularly interested in the persistency of the observed periods and phases, and techniques have been devised that provide such analyses, including the Periodic Regression (Bliss, 1958) and the Cosinor (Halberg, 1965). The mathematical principles underlying harmonic analysis have been succinctly described by Pfeiffer (1961), LePage (1941), and Chapman and Bartels (1940).

The investigator of biological time series phenomena often encounters asymmetric responses during 24-hour periods. Such asymmetric curves have been reported by Winget et al. (1965) on DBT measurements in the chicken; Winget et al. (1968a) on DBT, HR, and LA in the chicken; Winget et al. (1968b) on DBT and HR in the primate, *Cebus albafrons*; Winget et al. (1968c) on DBT in the primate, *Macacca nemestrina*; Carlson (1968) on HR in astronauts in the Gemini-7 flight; and Kayser and Heusner (1967) on oxygen uptake in the deer mouse, *Peromyscus*.

This list is by no means intended to be exhaustive, but is cited merely to indicate the wide range of species and physiological parameters wherein asymmetric biorhythm data have been encountered. The observed data curves generally indicate a rapid rise of the parameter to a peak value, followed by a plateau and a rapid fall to minimal levels. Such curves are not readily amenable to being analyzed by harmonic analysis, since poor fits result unless a large number of harmonics are utilized. More importantly, harmonic analysis does not explain the results obtained, and higher order harmonics are difficult to interpret (Blackman and Tukey, 1958).

Mean responses of physiological parameters (e.g., DBT) taken over a number of time periods may be thought of as a wave form that is stationary in time or one that, when properly transformed, becomes stationary in time. This paper is concerned with the mathematical analysis of such wave forms and with the detailed description of the mathematical methods developed for this purpose. These methods center on a unique curve-fitting device that represents a synthesis of well-known mathematical techniques and utilizes the method of moments. The curve-fitting technique has wide applicability to the fitting of data where the unknown parameters of the function appear nonlinearly, so that the method of least-squares cannot be applied directly. However, the particular application of the method is based on solutions of Volterra's Integro-Differential Equation (VIDE) (Volterra, 1913a, 1913b, 1959). The mathematical basis will be presented in detail, and a worked example will be given, utilizing heart rate data obtained from a male chicken.

MATHEMATICAL METHODS

Development of the Fundamental Period

Heart rate data were automatically collected on punched paper tape and were then subjected to harmonic analysis, using a computer program developed at the laboratory. The major features of the computer program are a quality control system and computations of periodograms and correlograms.

A quality control system was deemed necessary to ensure accuracy of the data before they were subjected to further analysis. Extreme data points were eliminated and nonharmonic trends, linear and nonlinear, were removed. Data points were judged to be extreme when they fell outside phsyiologic limits. Trends were removed by the use of least-squares procedures. A moving phasogram utilizing a 24-hour period was developed to detect sudden shifts in phases, as well as a moving periodogram that detected shifts in amplitudes of periods under observation. The data were then used to compute periodograms and correlograms.

Periodogram analysis is widely used to indicate possibly significant periods while correlograms are computed to verify the results of the periodogram. These analyses were also used here to establish the period for the stationary wave form.

Analysis of the Wave Form

The curve-fitting technique to be described could be illustrated using many nonlinear functions beginning with the simple form, $y = a \exp(Kt)$. The function of interest here was the VIDE, which was utilized for illustrative purposes because of its wide applicability to biological processes. The mathematics are presented in detail for those interested in applying the technique to other exponential functions. A worked example will be given for those who wish to use the technique to fit their data but who are not interested in the detailed mathematics.

The VIDE may be written as follows:

$$\frac{1}{y}\frac{dy}{d\tau} = a - by + \int_{0}^{\tau} k(\tau,z)y(z)\,dz \qquad (1)$$

where y represents the value of the physiological system studied (in this case, heart rate), and where the three parameters represent (following Davis, 1962):

1. a, a generative factor proportional to the heart rate.
2. b, an inhibiting influence proportional to the square of the heart rate.
3. $k(\tau,z)$, a heredity component composed of the sum of individual factors encountered in the past.

These parameters will be developed from the data. It is necessary, however, to attempt to define these parameters in the present context as the terms "generative," "inhibiting," and "heredity" are not used in their usual sense. Volterra was concerned primarily with elucidating increases and decreases of numbers of individuals in animal populations, while Davis (1941) applied the VIDE to describing changes in economic systems, extrapolating from examples of growth of individual animals and of populations. Each of these authors could utilize the term "growth" in its accepted sense. In this paper the parameter, a, may be though of as a "growth" parameter in that it defines the rapidity at which heart rate increases, and the parameter, b, as a "decay" parameter in that it defines the rate at which heart rate is slowed. The third parameter, $k(\tau, z)$, may be thought of as a "memory" factor, i.e., a phenomenon representing the effect of past history on the heart rate of the animal. The biological meaning of these parameters will be discussed further.

The parameter $k(\tau, z)$ will be restricted to $k(\tau - z)$, since Volterra (1913a) has indicated that "in the case of a closed cycle, which is completely equivalent to the case where heredity is invariable, it can be shown that the integral equations are simply functions of the difference $(\tau - z)$ of the two variables" (see also Volterra, 1913b, 1959). The parameter $k(\tau - z)$ may be expanded mathematically into an infinite series of iterated integrals with factors of proportionality K_i. The first of these, K_o, relates the area under the wave form with the differential equation. Under the assumption that all the K_i, except K_o, are zero, and making the following transformations: $y = a\mu/b$; $\tau = x/a$; and $\lambda = K_o/ab$, where a and b are restricted to values greater than zero, equation (1) simplifies to:

$$\frac{1}{\mu}\frac{d\mu}{dx} = 1 - \mu + \lambda \int_o^x \mu dx \tag{2}$$

The family of curves produced by varying λ is shown in Fig. 1 (Davis, 1962). The case where $\lambda = 0$ generates the logistic or growth curve. If $\lambda > 0$, i.e., when K_o is positive, growth increases without limit. If $\lambda < 0$, unimodal types of curves are produced whose extreme values approach zero.

A wave form may be considered to represent the sum of several components, each of which may be of a type shown in Fig. 1. Hence, wave-form analysis consists primarily of partitioning the observed wave form into its components. An example of such partitioning has been given by Carver (1924) for actuarial data described by Pearson's frequency distribution system of curves.

The parameter, λ, must be obtained from the data, which implies that a, b, and K_o must be estimated. The techniques used for these estimations or, equivalently, for fitting the data curves will be described in detail.

Equation (1) may be rewritten in finite difference form (assuming b is negative):

$$\frac{1}{y(t+1)} \frac{\Delta y(t)}{\Delta t} = a + by(t) + \sum_{t=0}^{n-1} K[(n-1),-t]y(t) \tag{3}$$

where t is an assigned integer for the n data points at the times τ, i.e., $t = 0, 1, 2, \ldots, n-1, n$. For equally spaced data $\Delta t = 1$ and thus vanishes. Since $K[(n-1) - t]$ is a function of one variable, it may be written formally as follows, where Γ is the well-known gamma function:

$$K[(n-1) - t] = \frac{K_o}{\Gamma(1)} + \frac{K_1(n-1-t)}{\Gamma(2)} + \frac{K_2(n-1-t)^2}{\Gamma(3)} + \cdots \tag{4}$$

In order to transform the power term $(n-1-t)^S$ into its factorial representation $(n-1-t)^{(S)}$, the difference of zero, D_q^S, are utilized (Davis, 1962). Thus,

$$(n-1-t)^S = \sum_{q=0}^{S} D_q^S \frac{(n-1-t)^{(q)}}{\Gamma(q+1)} \tag{5}$$

Equation (4) is therefore:

$$K[(n-1)-t] = \sum_{S=0}^{\infty} K_S \left[\sum_{q=0}^{S} \frac{D_q^S (n-1-t)^{(q)}}{\Gamma(q+1)} \right] \tag{6}$$

where $(n-1-t)^{(q)} = (n-1-t)(n-2-t)(n-3-t)\ldots(n-q+1)$.

Substituting (6) into (3):

$$\frac{\Delta y(t)}{y(t+1)} = a + by(t) + \sum_{S=0}^{\infty} K_S \left[\sum_{q=0}^{S} D_q^S \sum_{t=0}^{n-1-q} \frac{(n-1-t)^{(q)}}{\Gamma(q+1)} y(t) \right] \tag{7}$$

and noting that $(n-1-t)^{(S)}$ vanishes for $t > (n-1-q)$, we obtain:

$$\Delta y(t) = ay(t+1) + by(t+1)y(t)$$

$$+ y(t+1) \sum_{S=0}^{\infty} K_S \left[\sum_{q=0}^{S} D_q^S \sum_{t=0}^{n-1-q} \frac{(n-1-q)^{(q)}}{\Gamma(q+1)} y(t) \right] \tag{8}$$

The summation operator, P_n^{-r}, of the finite difference calculus (Milne-Thompson, 1951), is analogous to the general integration operator, ${}_oD_t^{-m}$ (Davis, 1951) of the infinitesimal calculus and has a formula similar to:

$${}_oD_t^{-m} y(t) = \int_o^t dz \int_o^z dz \cdots \int_o^z y(z)\,dz = \frac{1}{\Gamma(m)} \int_o^t (t-z)^{m-1} y(z)\,dz \tag{9}$$

The finite difference formulation of the above is

$$P_n^{-r} y(t) = P_{(n-r+1)}^{-1} \left\{ \frac{\Gamma(n-t)\,y(t)}{\Gamma(r)\,\Gamma(n-t-r+1)} \right\} = P_{(n-r+1)}^{-1} \left\{ \frac{(n-t-1)^{(r-1)}}{\Gamma(r)} y(t) \right\} \tag{10}$$

Using this relationship we can rewrite equation (8) in terms of the P_n^{-r} operator, which, in turn, can be computed from the data.

$$\Delta y(t) = ay(t+1) + by(t+1)y(t) + y(t+1) \sum_{S=0}^{\infty} K_S \sum_{q=0}^{S} D_q^S P_n^{-(q+1)} y(t) \tag{11}$$

Summing equation (11) to form an integro-difference equation allows us to enter the value of y(0) (Hudson, 1953), which is lost in the formulation of difference-equation (11). After letting

$$\begin{aligned} G(t) &= y(t+1)t(t) \\ H(t,S) &= y(t+1) \sum_{q=0}^{S} D_q^S P_n^{-(q+1)} y(t) \end{aligned} \tag{12}$$

we obtain

$$y(t) = aP_n^{-1} y(t+1) + bP_n^{-1} G(t) + P_n^{-1} \sum_{S=0}^{\infty} K_S H(t,S) + \hat{y}(0) \tag{13}$$

the integro-difference equation that is to be fit to the data. The method of least-squares may be utilized for fitting. However, the method of moments was considered to be more advantageous for two reasons. First, the area under the wave form is conserved, and second, the actual fitting procedure is greatly simplified because the summation operators, P_n^{-r}, are calculated directly from the data (Hartley, 1948) and are related simply to the moments of functions calculated about a point, t = n-1.

In order to apply the method of moments (Elderton, 1953), we multiply the above equation by $(t-n+1)^r$ and sum from $t = 0$ to $n - 1$ to obtain:

$$P_n^{-1}(t-n+1)^r y(t) = aP_n^{-1}\left[(t-n+1)^r\ P_n^{-1}y(t+1)\right] + bP_n^{-1}\left[(t-n+1)^r\ P_n^{-1}G(t)\right]$$

$$+ \sum_{S=0}^{\infty} K_S\ P_n^{-1}\left[(t-n+1)^r\ P_n^{-1}H(t,S)\right] + \hat{y}(0)\ P_n^{-1}(t-n+1)^r \quad (14)$$

It can be shown that

$$P_n^{-1}(t-n+1)^r y(t) = (-1)^r \sum_{x=0}^{r} D_x^r\ P_n^{-(x+1)}y(t) \quad (15)$$

Then (14) may be rewritten as:

$$\sum_{x=0}^{r} D_x^r\ P_n^{-(x+1)}y(t) = a\sum_{x=0}^{r}\left[D_x^r\ P_n^{-(x+2)}y(t+1)\right] + b\sum_{x=0}^{r}\left[D_x^r\ P_n^{-(x+2)}G(t)\right]$$

$$+ \sum_{S=0}^{\infty} K_S \sum_{x=0}^{r}\left[D_x^r\ P_n^{-(x+2)}H(t,S)\right]$$

$$+ \hat{y}(0) \sum_{x=0}^{r}\left[D_x^r\ \frac{\Gamma(n+1)}{\Gamma(x+2)\Gamma(n-x)}\right] \quad (16)$$

Setting $r = 0, 1, 2, 3 \ldots$ any number of simultaneous linear equations may be developed whose solution provides estimates of the parameters a, b, K_S, and $y(0)$.

We have been able to describe the present data with $K(\tau,z)$ limited to the first term of its expansion, K_o. If this is done, we obtain:

$$\sum_{x=0}^{r} D_x^r P_n^{-(x+1)} y(t) = a \sum_{x=0}^{r} \left[D_x^r P_n^{-(x+2)} y(t+1) \right] + b \sum_{x=0}^{r} \left[D_x^r P_n^{-(x+2)} G(t) \right]$$

$$+ K_o \sum_{x=0}^{r} \left[D_x^r P_n^{-(x+2)} H(t,0) \right]$$

$$+ \hat{y}(0) \sum_{x=0}^{r} \left[D_x^r \frac{\Gamma(n+1)}{\Gamma(x+2)\Gamma(n-x)} \right] \qquad (17)$$

The D_x^r or differences of zero (equation (5)) are given in Table I.

RESULTS

A mature, male, Single Comb White Leghorn chicken was confined in a cage maintained under thermally neutral conditions and was given 14 hours of light (0600-2000 hr) and 10 hours of darkness (2000-0600 hr). Heart rate data were collected at 6-minute intervals over a 14-day time period, following a prior equilibration period. Part of the data are shown in Fig. 2, in which the wave form is readily seen.

The 6-minute means, suitably edited as described earlier, were utilized to produce a periodogram, Fig. 3, and a correlogram, Fig. 4.

The significance of individual periods was tested by use of Fisher's probability distribution (Fisher, 1929). Significant periods were observed at 24, 12, 6, and 4 hours, $P < 0.001$, with a suggestion of a broad band at 8 hours. The correlogram, Fig. 4, verified the significance of the 24-hour period.

The phase of the 24-hour harmonic was consistent over the 14-day data collection period as shown by the "moving phaseogram" analysis. The lack of change of the phase permitted the 14-day data to be pooled for wave-form analysis.

Mean heart rates at hourly intervals were then computed for the 14-day period and were normalized by dividing the mean of hourly means by the grand mean. These "normalized" means were utilized exclusively in the analyses to be discussed.

Harmonic Analysis

Harmonic fits to the normalized hourly mean heart rates were computed and are shown in Figs. 5 and 6. The 24-hour harmonic clearly did not provide a good fit to the data (Fig. 5). A much better fit was obtained when harmonics corresponding to 24, 12, 8, 6, and 4 hour periods were employed (Fig. 6).

Wave-Form Analysis

The hourly mean heart rates shown in Fig. 5 were asymmetrically distributed. The minimum heart rate was reached at 2000 hr, and the maximum at 0700 hr. The rapid rise and fall of heart rates preceded the lights being turned on and off (0600 and 2000 hr). The data from 1400 to 2200 hr appeared to follow a logistic or "growth" curve. The data from 2200 to 1400 hr appeared to be a composite of two curves, a unimodal and a logistic. It was, therefore, possible to analyze this portion of the total curve in two ways: Hypothesis I: a logistic beginning at 2200 hr to which a unimodal is added later; and Hypothesis II: a unimodal beginning at 2200 hr and a logistic added later. Curves were fit under both hypotheses, although only the fitting of the logistic and unimodal under Hypothesis I will be presented in detail.

THE WORKED EXAMPLE

Table II contains the observed (normalized) data and the fitted values for the first and second approximations. The observed data (column 2) had been normalized by dividing each hourly mean by the grand mean. These values were coded for working purposes by subtracting from each the minimal or basal value of 94.2 and then multiplying by 10 to obtain integer values (column 3). The sum of the coded values was 1409, which is proportional to the area under the curve of normalized means.

Fitting the Logistic

Three working postulates were utilized:

1. The starting time was taken at 2200 hr.
2. The maximum value was taken at 80 (coded data), which is the average of the plateau (1000-1500 hr).
3. The logistic is symmetrical about its inflection point, which, in this case, is halfway between 0 at 2200 hr and 80 at 0600 hr, at which time the maximum was first obtained.

The first approximation to the logistic was blocked out by hand, with values as shown in column 4 of Table II. These values were the result of several estimations, the final values chosen to produce a unimodal curve, when the estimated values were subtracted from the coded values. The estimated values, $y(t)$, were utilized as shown in Table III. The formation of the summation operators (e.g., $P_n^{-1} y(t)$) can be easily seen. It may be noted that each value in any of the summation columns is the sum of the values behind it. Thus,

$$P_5^{-1} y(t) = 102 = \sum_{t=0}^{4} y(t)$$

In developing the set of simultaneous equations whose solution provides the estimates of a, b, and y(0), the parameters of the logistic, only the final values of the summation columns are used. It should be recalled that for the logistic $K_o = 0$, hence only three simultaneous equations need be solved.

The simultaneous equations are, following equation (17):

$$906a + 34900b + 8y(0) = 325$$

$$1203a + 29655b + 28y(0) = 597$$

$$[1203 + 2(1040)]a + [29655 + 2(15600)]b + 140y(0) = [597 + 2(662)]$$

In final form:

$$906a + 34900b + 8y(0) = 325$$

$$1203a + 29655b + 28y(0) = 597$$

$$3283a + 60855b + 140y(0) = 1921$$

It may be seen that the first three successive coefficients of y(0) are n, $[n(n-1)]/2$, and $[n(n-1)(2n-1)]/6$. The fourth coefficient required in fitting the unimodal is given by $[n(n-1)/2]^2$.

The solutions of these simultaneous equations, obtained in the usual fashion, are:

$$\hat{a} = 0.6344, \quad \hat{b} = -0.0077, \text{ and } \hat{y}(0) = 2.1724$$

The recursion relationship that estimates the successive fitted values (upper half of column 7 of Table II) is given by

$$\hat{y}(t+1) = \frac{\hat{y}(t)}{(1-\hat{a}) - \hat{b}y(t)} \quad (18)$$

which, in this case is

$$\hat{y}(t+1) = \frac{\hat{y}(t)}{0.3656 + 0.0077\ \hat{y}(t)}$$

beginning with $\hat{y}(0) = 2.1724$.

Fitting the Unimodal

The estimation of the total curve from 2200 to 1400 hr is shown in Table II, column 6. The estimates of the logistic were subtracted from the total curve to provide the first approximation of the unimodal. The development of the summation operators is as shown in Table IV as are the coefficients of the parameters to be estimated, a, b, K_o, and y (0). Equation (17), applicable to the unimodal curve, may be written in matrix notation AC = R where the elements of the matrices are developed as shown in the latter part of Table IV. The C matrix is the desired solution, a, b, K_o, and y(0) and is found by solving $C = A^{-1}R$. The details are as follows:

$$A = \begin{vmatrix} 1199 & 35090 & 48758 & 10 \\ 3224 & 84941 & 91480 & 45 \\ 13566 & 318127 & 287692 & 285 \\ 66812 & 1379807 & 1069198 & 2025 \end{vmatrix}$$

$$R = \begin{vmatrix} 206 \\ 1013 \\ 5615 \\ 33479 \end{vmatrix} \qquad A^{-1} = \begin{vmatrix} -0.0009 & 0.0012 & -0.0003 & 0.0000+ \\ 0.0188 & -0.0030 & 0.0088 & -0.0007 \\ 0.0002 & -0.0002 & 0.0000+ & -0.0000+ \\ -0.1431 & 0.2581 & -0.0859 & 0.0076 \end{vmatrix}$$

$$C = \begin{vmatrix} a \\ b \\ K_o \\ y(0) \end{vmatrix} \qquad C = A^{-1}R = \begin{vmatrix} 0.6874 \\ -0.0058 \\ -0.0090 \\ 2.5760 \end{vmatrix}$$

The recursion relationship to develop the successive values of y(t) is:

$$\hat{y}(t+1) = \frac{\hat{y}(t)}{(1-\hat{a}) - \hat{b}\hat{y}(t) - \hat{K}_o P_t^{-1}\hat{y}(t)} \tag{19}$$

where $P_t^{-1}\hat{y}(t)$ are the successive values of $P_n^{-1}\hat{y}(t)$, beginning with $P^{-1}\hat{y}(t) = 0$. The first value y(0) = 2.5760; the second would be given by:

$$\hat{y}(1) = \frac{\hat{y}(0)}{(1-\hat{a}) - \hat{b}\hat{y}(0)}$$

The fitted values for y(t) are shown in column 8 of Table II.

The descending logistic from 1400-2100 hr was fit identically under each hypothesis in a manner similar to that described for the ascending logistic given above. The backward relationship for a logistic is:

$$\hat{y}(t) = \frac{\hat{y}(t+1)}{[\hat{b}/(1-\hat{a})]\hat{y}(t+1) + [1/(1-\hat{a})]} \tag{20}$$

Equations (18) and (20) permit "forward" and "backward" solutions of the logistic equation. It is thus possible to utilize data covering only a portion of the logistic curve to estimate the necessary parameters (a, b, and y(0)) and to extend the fitted curve in either or both directions. In this case, equation (20) was utilized (backward solution), although equation (18) could have been as easily used.

The logistic and unimodal under Hypothesis II were fit similarly. The estimated parameters for all five curves are shown in Table V. The total curves and their components fit under the two hypotheses are shown in Figs. 7 and 8.

DISCUSSION

The techniques of wave-form analysis are intended not to replace, but rather to supplement harmonic analysis. Wave-form analysis begins only after harmonic analysis has been completed and helps define functional relationships in ways that would be quite difficult were only harmonic analysis used. Asymmetric data, such as daily heart rate variation in the chicken, were indeed fit with a harmonic series (Fig. 6), but the fit represented an empirical relationship in that the parameters (harmonics) could not be readily utilized to explain the observed data. On the other hand, the VIDE is a biologically-derived model containing "growth" and "inhibitory" parameters that relate to real, but as yet unidentified, physicochemical processes involved in establishing the observed cycles.

In discussion of the VIDE it is not possible at this time to utilize standard terminology in precise ways, as the necessary experimental work has yet to be done. Basic concepts, however, should not be ignored simply because they cannot be defined precisely. It is conceptually correct to speak of the area under the wave form as being proportional to energy or work, even though the amount of work cannot be quantified. It is clear that the heart muscle has performed work. It is also evident that in the normal physiological situation, the greater the total number of beats per 24 hours, the greater the work performed and the greater the area under the wave form. It may thus be quite reasonable to attempt to define the parameters of the VIDE in terms of energy expenditures.

The "growth" parameter, a, is proportional to the energy inputs relative to the working of the heart, while b controls the rate at which energy is expended. The first is proportional to the heart rate, while the second is proportional to the square of the heart rate. In the logistic equation, b does not become influential until some time after the curve has lifted off the basal level. The mechanism(s) by which b operates is, of course, not known.

The unimodal curve contains, in addition to a and b, a third parameter, K_o. It is this parameter, the "memory," that causes the VIDE to operate, in Volterra's words, as a "closed cycle," thus ensuring the cyclic nature of the curve. The presence of this parameter infers that built into the system is a monitor that keeps track of the total amount of energy expended to any instant in time. At the same time the memory also controls the direction and path of the curve, so that the curve returns to its starting point, making possible the periodicity of the wave form.

The mathematical technique used to fit the VIDE family of equations was the method of moments. The criterion of "best fits" is the conservation of the area under the wave form, analogous to conservation of energy. By definition, the method of moments conserves the area under the wave form, so that areas under observed and fitted wave forms are identical, except for round-off error. Thus, the VIDE curves fit under Hypotheses I and II (Figs. 7 and 8) cannot be distinguished as to a better fit to the data, because the areas under each of the curves are, to all intents and purposes, the same.

Whether the curves fit under Hypothesis I are a more correct representation of the true physiological situation than are those fit under Hypothesis II cannot be ascertained mathematically. The estimated parameters (Table V) indicate that Hypothesis I may be more acceptable. First, the ascending logistic of Hypothesis I is more similar to the common, descending logistic, in that the parameters are more nearly alike than under Hypothesis II, arguing from the basis of physiological symmetry. Second, the "inhibitor," b, of the unimodal fit under Hypothesis II is positive, indicating that it is another energy input rather than an inhibitor, forcing the "memory," K_o, to serve as the inhibitor.

Mathematical reasoning must give way to physiological experimentation in order to properly distinguish between these hypotheses. It is not known what physiological parameters or mechanisms are represented by the component curves, nor is it known whether the logistic and the unimodal curves each represent a discrete parameter or are, in fact, each the sum of many logistic and unimodal curves, respectively.

The fitted curves, Figs. 7 and 8, give evidence of the entrainment of HR to the 14:10 light:dark regimen. Figure 7 (Hypothesis I) shows that the curve crosses the mean (100%, right-hand axis) at 0300 and 1700 hr, a 14:10 ratio. Also, the maximum and minimum points occur at 0700 and 2200 hr, again a 14:10 ratio. For the curves fit under Hypothesis II, similar ratios are seen. Winget et al. (1968b) observed entrainment of DBT and HR in the primate, Cebus albafrons, to a 12:12 light:dark regimen, based also on fitted VIDE curves.

Under Hypothesis I (Fig. 7) the liftoff of the first logistic from basal level occurs after 2200 hr, while that of the unimodal occurs after 0200 hr. The second logistic leaves its upper plateau after 1500 hr. Mathematically, since the logistic curve must remain at its lower or upper asymptote and the unimodal must remain at its basal level until perturbed, the presence of positive and negative pulses or signals is thus demonstrated. In fact, the pulses themselves are sufficient conditions for the establishment of the wave form, stationary in time.

The differences in time between the observed pairs of pulses approximate those times observed for significant periods in the periodogram (Fig. 3). The interval from 2200 to 0200 hr is 4 hours; that from 1500 to 2200 hr is 7 hours (approximately 6 hours); and that from any pulse to itself is 24 hours. The harmonic model assumes that significant periods repeat regularly, so that, for example, the 4-hour period occurs six times per 24-hour interval. However, the contributions to the sum of harmonics made by each period are not equal over the 24-hour interval, and, in fact, the contribution may be either negative or positive. Biologically, a regular 4-hour period may be unrealistic, yet it may be utilized by harmonic analysis to better fit a portion of the wave form. In the harmonic model only a single pulse per harmonic is required, one that could have occurred at any time in the past. In the VIDE model a pulse is required at least once within the basic period, in this case, every 24 hours. It is therefore postulated that the intervals between pulses may be picked up by harmonic analysis as significant periods, even though they do not recur within the basic period.

The VIDE model may be more functionally related to the true physiological mechanisms, and may thus provide the biologist with opportunities to understand biorhythm data. The VIDE model by itself does not indicate which of the three pulses noted is the primary pulse (and hence leads) and which pulses lag. The primary pulse may be thought of as the phasengeber and perhaps can be distinguished by experiments that are designed to compare relative degrees of entrainment to the photoperiod.

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Table I DIFFERENCES OF ZERO

x \ r	1	2	3	4	5	6
1	1	1	1	1	1	1
2		2	6	14	30	62
3			6	36	150	540
4				24	240	1560
5					120	1800
6						720

This table may be extended by the finite difference formulae:

$$x < r:\ D_x^r = xD_x^{r-1} + xD_{x-1}^{r-1}$$

$$x = r:\ D_x^r = \Gamma(r+1) = r!$$

$$x > r:\ D_x^r = 0$$

Table II CODED NORMALIZED HEART RATE MEANS OBSERVED AND ESTIMATED UNDER HYPOTHESIS I

			First Approximation (Freehand)			Second Approximation (Mathematical Fit)		
Hour	Normalized Data	Coded Data[1]	Logistic[2]	Unimodal	Total	Logistic[2]	Unimodal	Total
2200	94.6	4	0		0	0		0
2300	94.6	4	2		2	2		2
2400	95.7	15	5		5	6		6
0100	96.9	27	15		15	14		14
0200	97.1	29	30		30	29		29
0300	97.6	34	50	2	52	50	3	53
0400	100.4	62	65	9	74	66	8	74
0500	105.0	108	78	27	105	76	21	97
0600	106.1	119	80	42	122	80	39	119
0700	107.3	131	(80)	49	129	(80)	48	128
0800	105.3	111	(80)	35	115	(80)	39	119
0900	104.9	107	(80)	23	103	(80)	24	104
1000	102.9	87	(80)	11	91	(80)	13	93
1100	101.7	75	(80)	6	86	(80)	6	86
1200	102.7	85	(80)	2	82	(80)	3	83
1300	101.7	75	(80)		80	(80)	1	81
1400	102.7	85	80		80	80		80
1500	101.8	76	78		78	78		78
1600	100.5	63	74		74	73		73
1700	100.4	62	60		60	61		61
1800	98.0	38	38		38	40		40
1900	94.7	5	19		19	18		18
2000	92.7	5(-15)	6		6	7		7
2100	94.4	2	2		2	2		2
Sum		1409			1448			1447

[1]Value in parentheses is the observed coded value. A value of 5 was substituted.
[2]Values in parentheses are on the upper plateau, assumed, but not used in fitting the logistic.

Table III FIT OF THE FIRST LOGISTIC UNDER HYPOTHESIS I

t	Y(t)	$P_n^{-1}y(t)$	$P_n^{-2}y(t)$	$P_n^{-3}y(t)$	y(t+1)	$P_n^{-1}y(t+1)$	$P_n^{-2}y(t+1)$	$P_n^{-3}y(t+1)$	$P_n^{-4}y(t+1)$
0	2	0	0	0	5	0	0	0	0
1	5	2	0	0	15	5	0	0	0
2	15	7	2	0	30	20	5	0	0
3	30	22	9	2	50	50	25	5	0
4	50	52	31	11	65	100	75	30	5
5	65	102	83	42	78	165	175	105	35
6	78	167	185	125	80	243	340	280	140
7	80	245	352	310		323	583	620	420
8		325	597	662			906	1203	1040

G(t) =

t	y(t)y(t+1)	$P_n^{-1}G(t)$	$P_n^{-2}G(t)$	$P_n^{-3}G(t)$	$P_n^{-4}G(t)$	$\hat{y}(t)$
0	10	0	0	0	0	2.1724
1	75	10	0	0	0	5.6824
2	450	85	10	0	0	13.8798
3	1500	535	95	10	0	29.3752
4	3250	2035	630	105	10	49.6370
5	5070	5285	2665	735	115	66.3773
6	6240	10355	7950	3400	850	75.7126
7	6400	16595	18305	11350	4250	79.8150
8		22995	34900	29655	15600	

Table IV FIT OF THE UNIMODAL UNDER HYPOTHESIS I

t	$y(t)$	$P_n^{-1}y(t)$	$P_n^{-2}y(t)$	$P_n^{-3}y(t)$	$P_n^{-4}y(t)$	$y(t+1)$	$P_n^{-1}y(t+1)$	$P_n^{-2}y(t+1)$	$P_n^{-3}y(t+1)$	$P_n^{-4}y(t+1)$	$P_n^{-5}y(t+1)$
0	2	0	0	0	0	9	0	0	0	0	0
1	9	2	0	0	0	27	9	0	0	0	0
2	27	11	2	0	0	42	36	9	0	0	0
3	42	38	13	2	0	49	78	45	9	0	0
4	49	80	51	15	2	35	127	123	54	9	0
5	35	129	131	66	17	23	162	250	177	63	9
6	23	164	260	197	83	11	185	412	427	240	72
7	11	187	424	457	280	6	196	597	839	667	312
8	6	198	611	881	737	2	202	793	1436	1506	979
9	2	204	809	492	1618	0	204	995	2229	2942	2485
10		206	1013	2301	3110			1199	3224	5171	5427

$G(t) = y(t)y(t+1)$ $\qquad$ $H(t) = y(t+1)P_n^{-1}y(t)$

$G(t)$	$P_n^{-1}G(t)$	$P_n^{-2}G(t)$	$P_n^{-3}G(t)$	$P_n^{-4}G(t)$	$P_n^{-5}G(t)$	$H(t)$	$P_n^{-1}H(t)$	$P_n^{-2}H(t)$	$P_n^{-3}H(t)$	$P_n^{-4}H(t)$	$P_n^{-5}H(t)$
18	0	0	0	0	0	0	0	0	0	0	0
243	18	0	0	0	0	54	0	0	0	0	0
1134	261	18	0	0	0	462	54	0	0	0	0
2058	1395	279	18	0	0	1862	516	54	0	0	0
1715	3453	1674	297	18	0	2800	2378	570	54	0	0
805	5168	5127	1971	315	18	2967	5178	2948	624	54	0
253	5973	10295	7098	2286	333	1804	8145	8126	3572	678	54
66	6226	16268	17393	9384	2619	1122	9949	16271	11698	4250	732
12	6292	22494	33661	26777	12003	396	11071	26220	27969	15948	4982
	6304	28786	56155	60438	38730		11467	37291	54189	43917	20930
		35090	84941	116693	99218			48758	91480	98106	64847

$R_1 =$ $= 206$

$R_2 =$ $= 1013$

$R_3 = 1013 + 2(2301)$ $= 5615$

$R_4 = 1013 + 6(2301) + 6(3110)$ $= 33479$

$A_{11} =$ $= 1199$

$A_{12} =$ $= 3224$

$A_{13} = 3224 + 2(5171)$ $= 13566$

$A_{14} = 3224 + 6(5171) + 6(5427)$ $= 66812$

$A_{12} =$ $= 35090$

$A_{22} =$ $= 84941$

$A_{32} = 84941 + 2(11653)$ $= 318127$

$A_{42} = 84941 + 6(11653) + 6(99218)$ $= 1379807$

$A_{13} =$ $= 48758$

$A_{23} =$ $= 91480$

$A_{33} = 91480 + 2(98106)$ $= 287692$

$A_{43} = 91480 + 6(98106) + 6(64847)$ $= 1069198$

$A_{14} = n$ $= 10$

$A_{24} = n(n-1)/2$ $= 45$

$A_{34} = n(n-1)(2n-1)/6$ $= 285$

$A_{44} = (A24)^2$ $= 2025$

Table V ESTIMATED PARAMETERS OF THE FITTED CURVES

Hypothesis	Type of Curve	Hours	$\hat{a}$	$\hat{b}$	$\hat{K}_o$	$\hat{y}(o)$
I	Logistic	2300 - 0600	0.6344	-0.0077	—	2.7124
	Unimodal	0300 - 1300	0.6874	-0.0058	-0.0090	2.5760
II	Unimodal	2200 - 1300	0.3883	0.0042	-0.0038	3.2970
	Logistic	0400 - 1000	0.8196	-0.0103	—	0.8196
I and II	Inverted Logistic	1400 - 2100	0.6966	-0.0087	—	2.1156

FIGURE CAPTIONS

Figure 1 Parametric set of solutions of $\frac{1}{\mu}\frac{d\mu}{dx} = 1 - \mu + \lambda \int_{o}^{x} \mu dx$ in terms of λ.

Figure 2 Heart rate data collected at 6-minute intervals over a 14-day time period. Shaded area of wave form indicates hours of darkness in the 24-hour period.

Figure 3 Periodogram of heart rate from data collected from an isolated male chicken. K is the square of the amplitude (R) of the oscillation normalized by the length (N) and variance (σ^2) of the time series ($K = NR^2/4\sigma^2$).

Figure 4 Correlogram of heart rate from data collected every 0.1 hour from an isolated male bird for 14 consecutive days. The correlation coefficient is for lag k (i.e., when k = 1, lag = 0.1 hour; therefore, k = 240 represents a lag of 24 hours).

Figure 5 A harmonic fit (τ = 24 hours) to the normalized hourly mean heart rates from an isolated male chicken.

Figure 6 Harmonic fits (τ = 24, 12, 8, 6, and 4 hours) to the normalized hourly mean heart rate from an isolated male chicken.

Figure 7 The VIDE curve fit with Hypothesis I.

Figure 8 The VIDE curve fit with Hypothesis II.

$$\frac{1}{\mu}\frac{d\mu}{dx} = 1 - \mu + \lambda \int_0^x \mu \, dx \text{ IN TERMS OF } \lambda$$

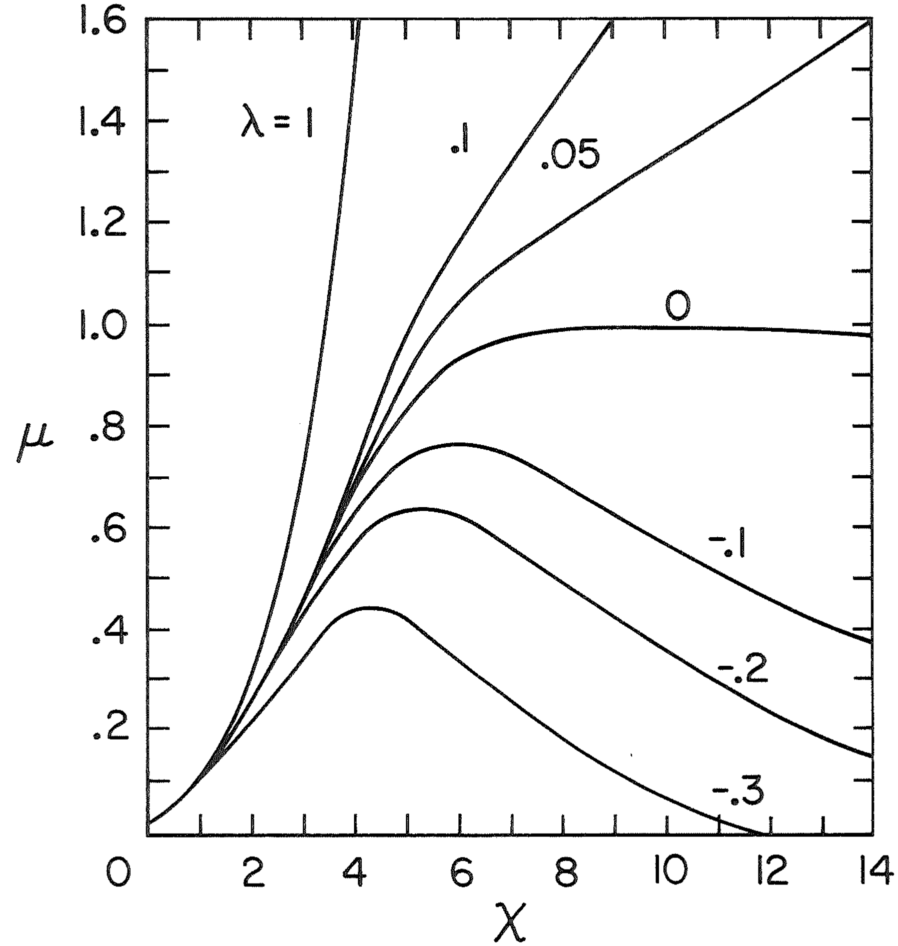


Figure 1.

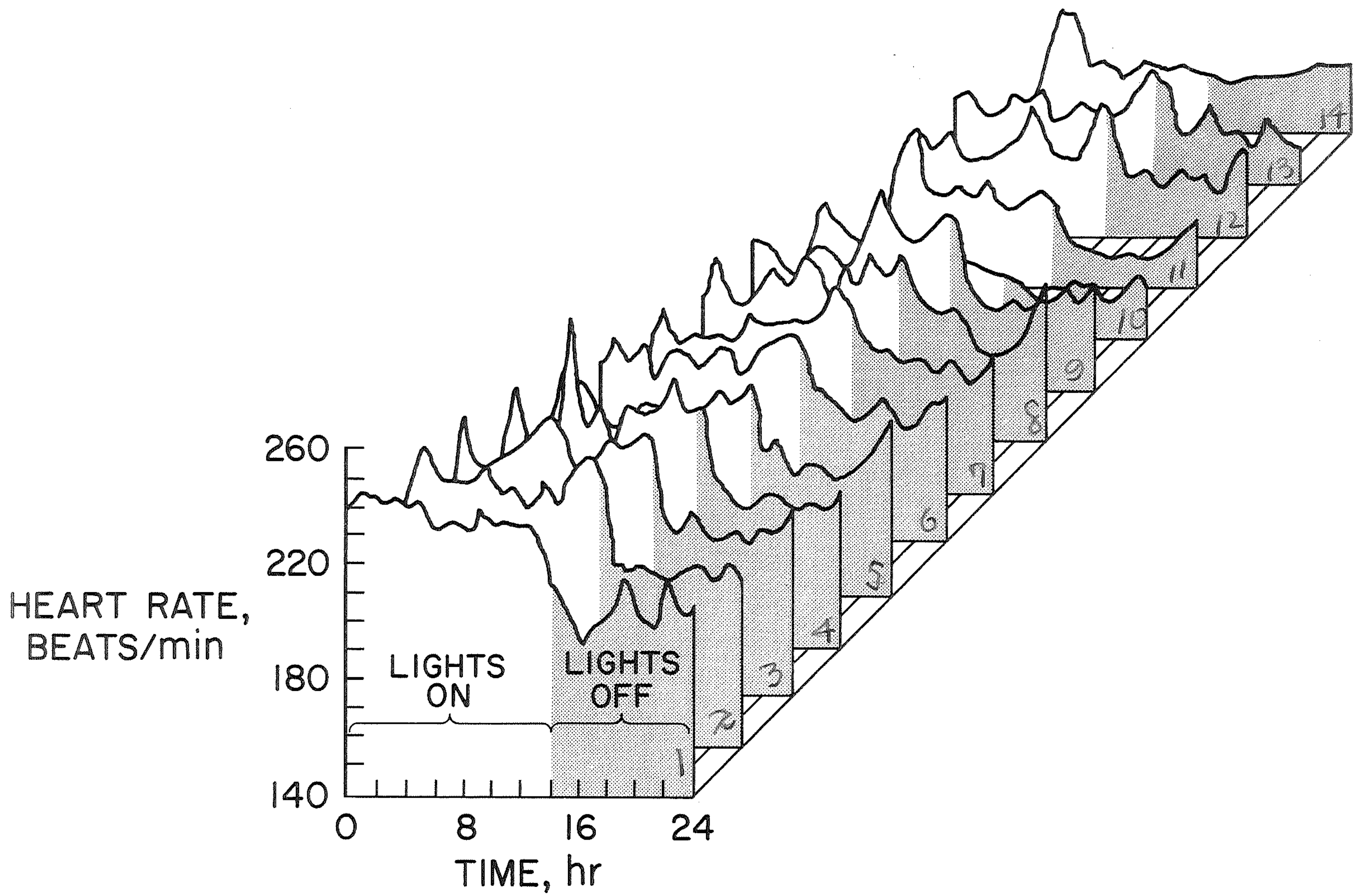

HEART RATE, BEATS/min
260
220
180
140
LIGHTS ON
LIGHTS OFF
0
8
16
24
TIME, hr
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 2.

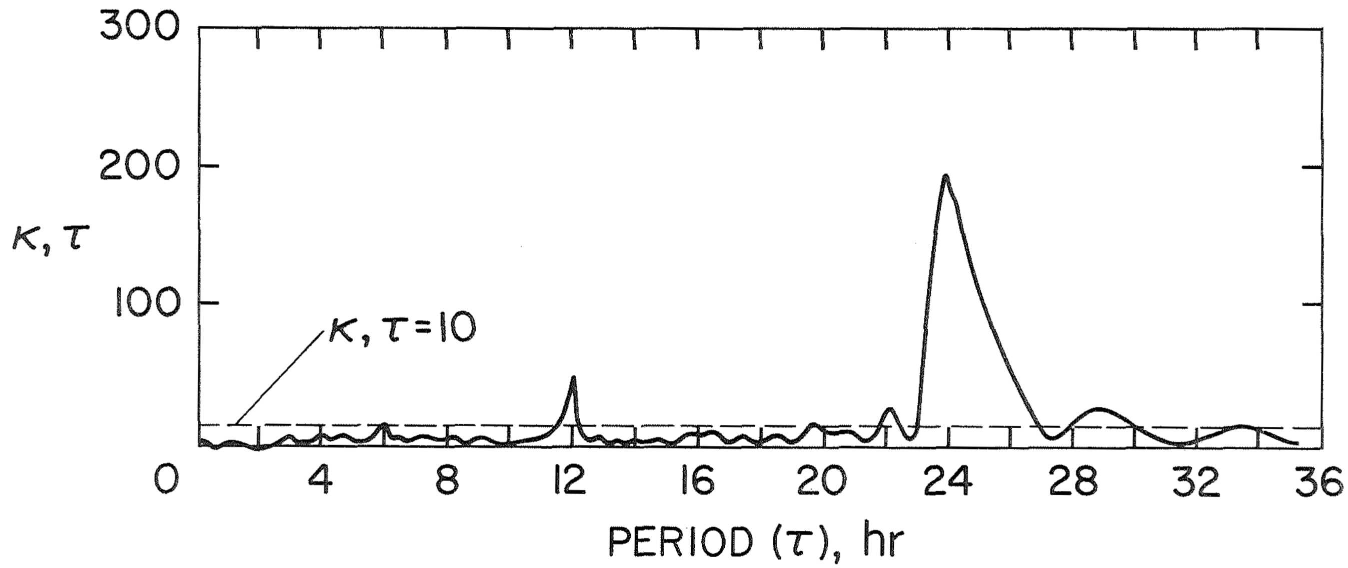
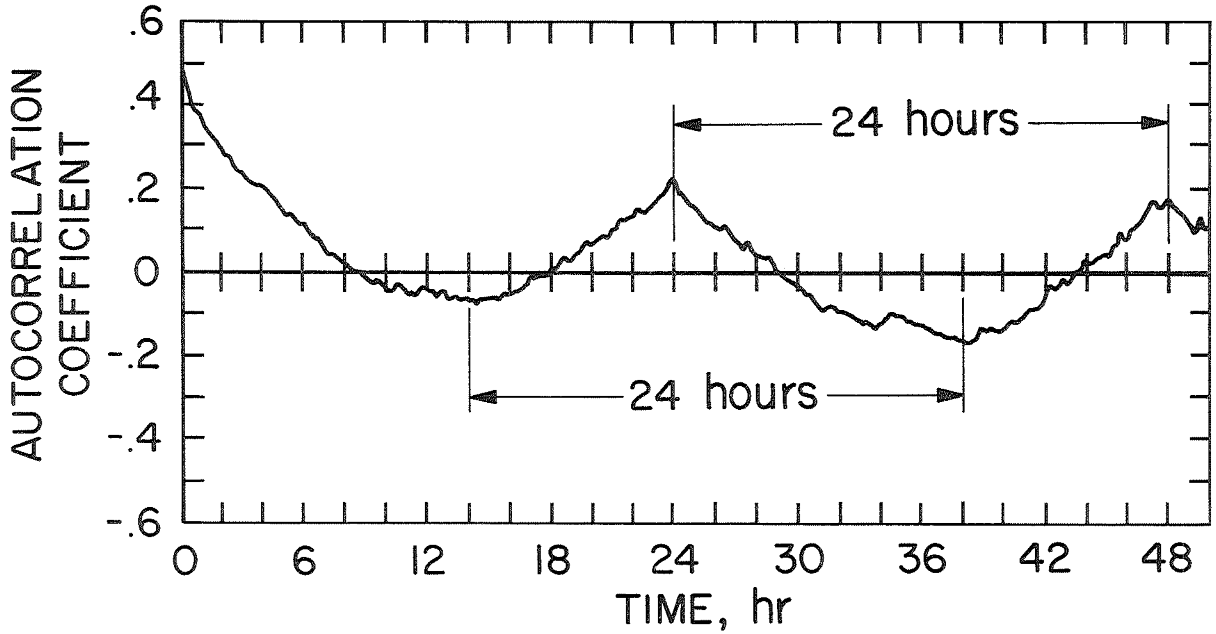


Figure 3.



AUTOCORRELATION COEFFICIENT
.6
.4
.2
0
-.2
-.4
-.6
24 hours
24 hours
0
6
12
18
24
30
36
42
48
TIME, hr

Figure 4.

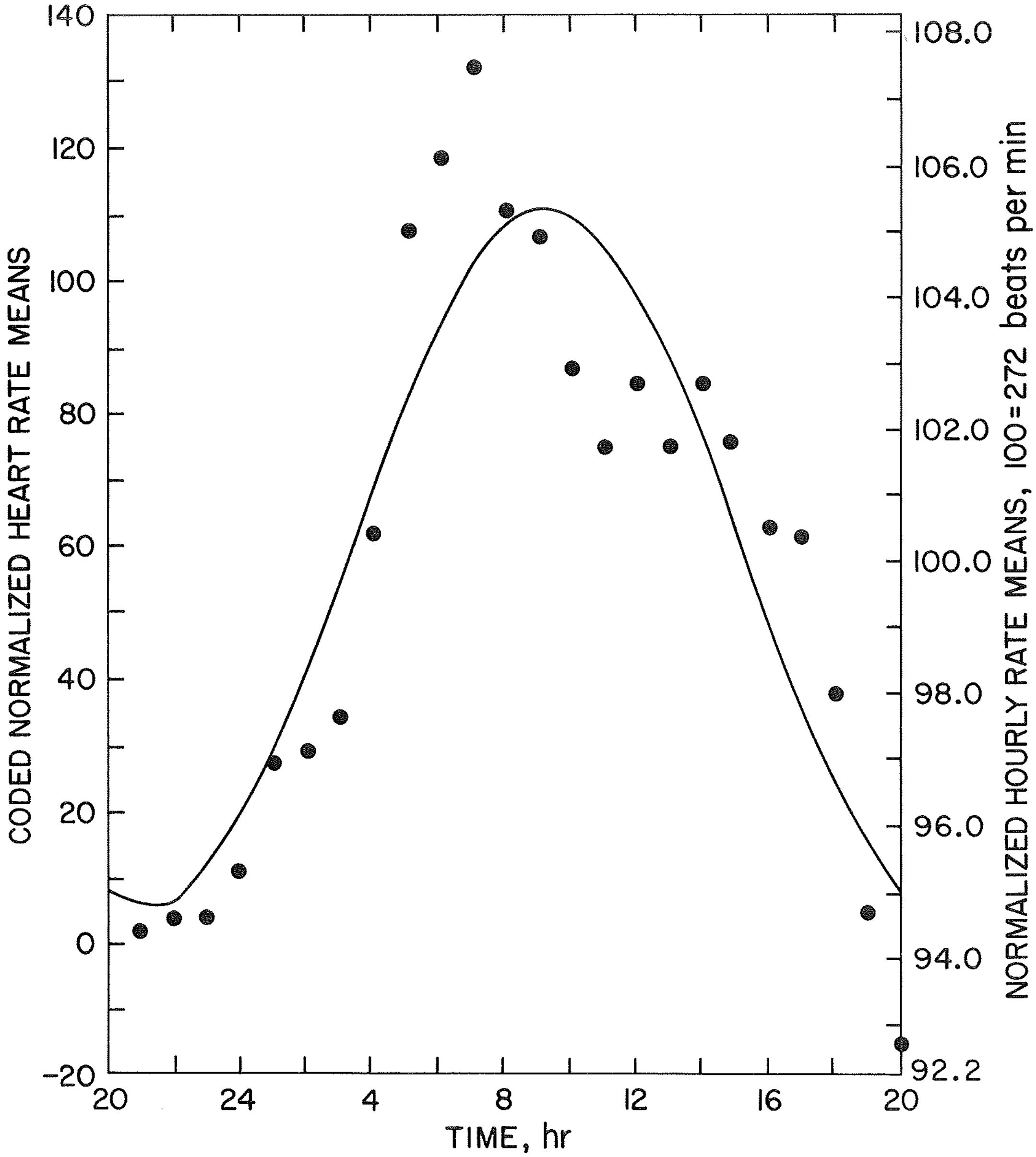


Figure 5.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA

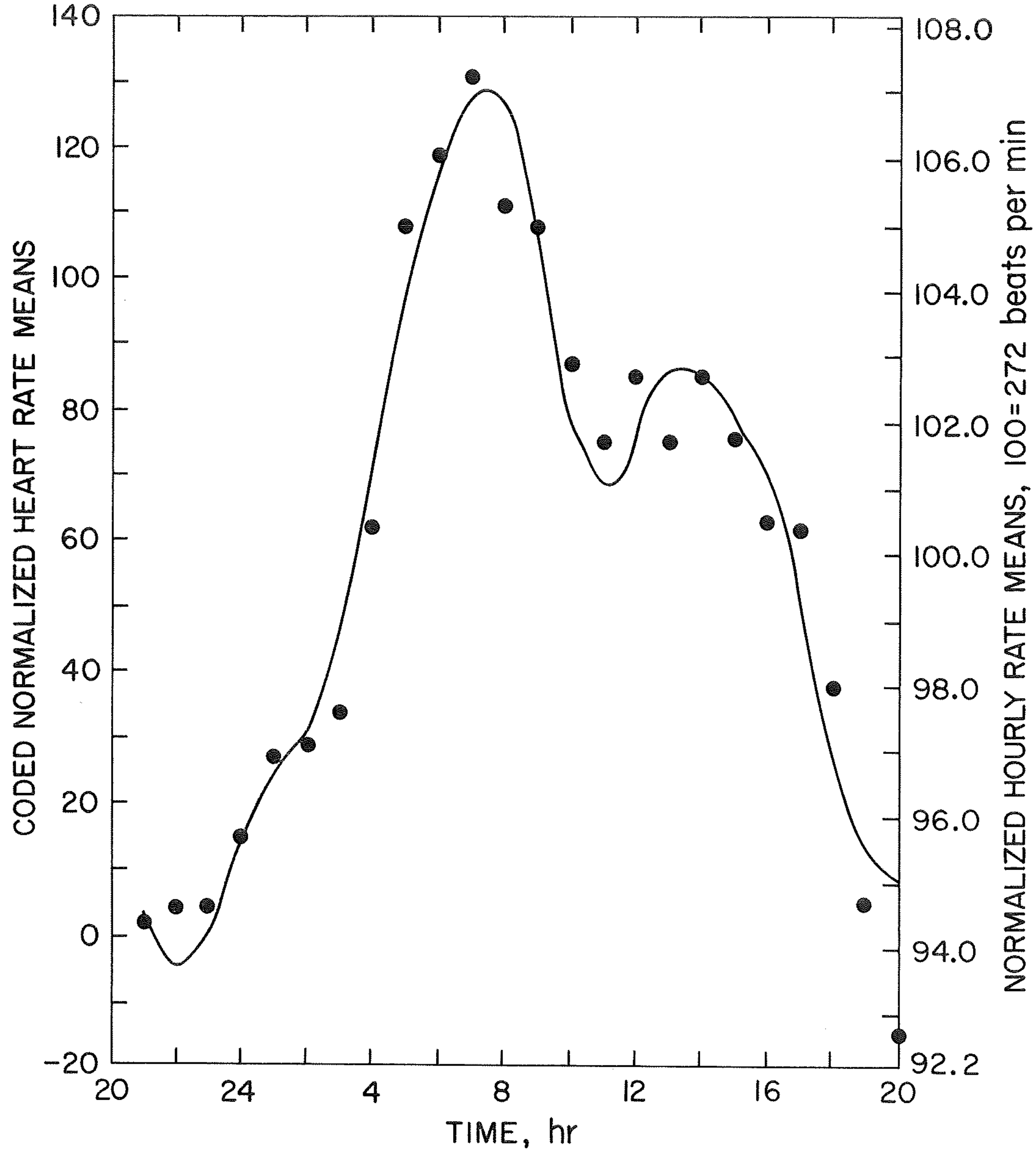

140
120
100
80
60
40
20
0
-20
108.0
106.0
104.0
102.0
100.0
98.0
96.0
94.0
92.2
20
24
4
8
12
16
20
TIME, hr
CODED NORMALIZED HEART RATE MEANS
NORMALIZED HOURLY RATE MEANS, 100=272 beats per min

Figure 6.

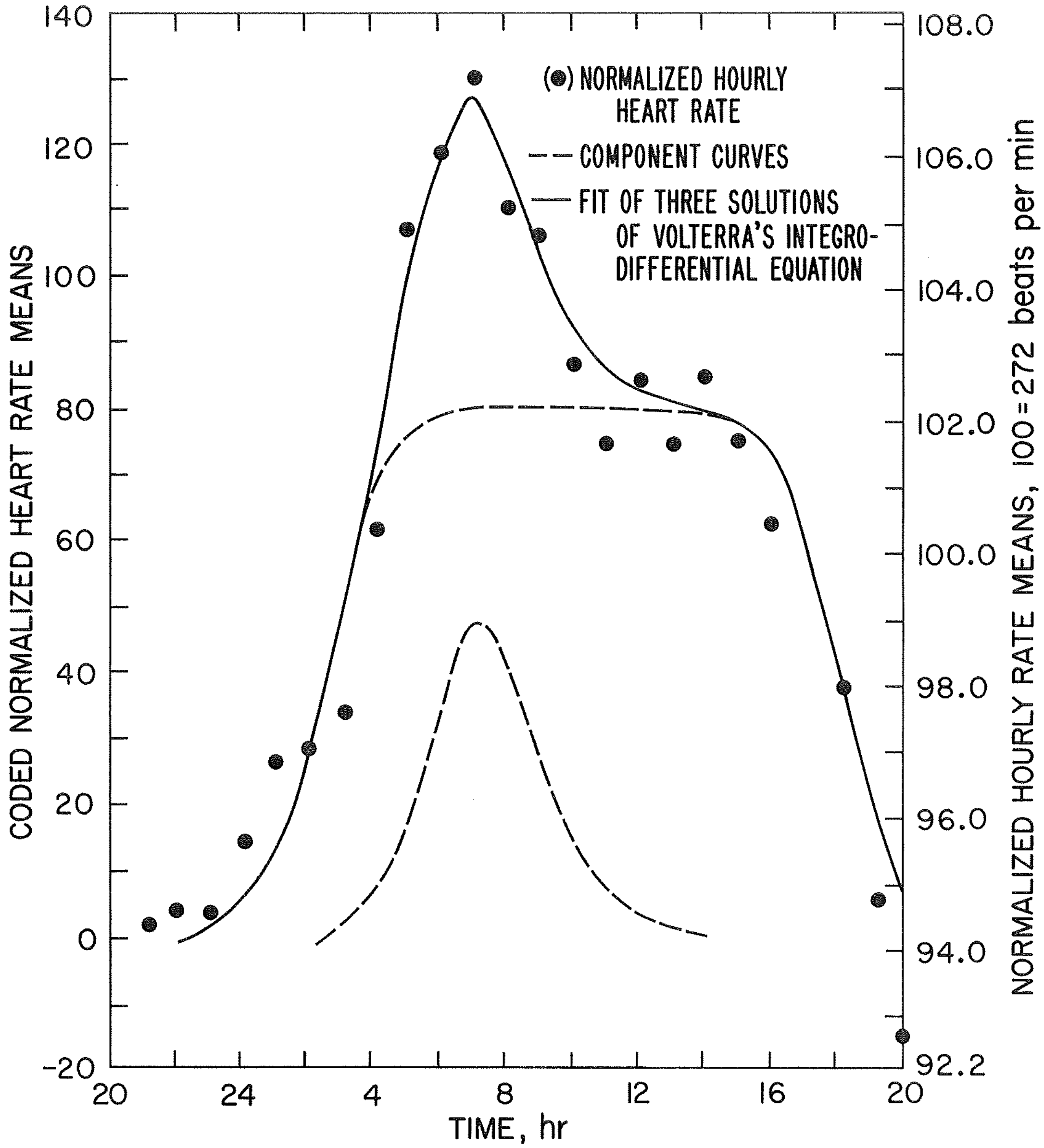

140
120
100
80
60
40
20
0
-20
108.0
106.0
104.0
102.0
100.0
98.0
96.0
94.0
92.2
20
24
4
8
12
16
20
TIME, hr
CODED NORMALIZED HEART RATE MEANS
NORMALIZED HOURLY RATE MEANS, 100=272 beats per min
(●) NORMALIZED HOURLY HEART RATE
COMPONENT CURVES
FIT OF THREE SOLUTIONS OF VOLTERRA'S INTEGRO-DIFFERENTIAL EQUATION

Figure 7.

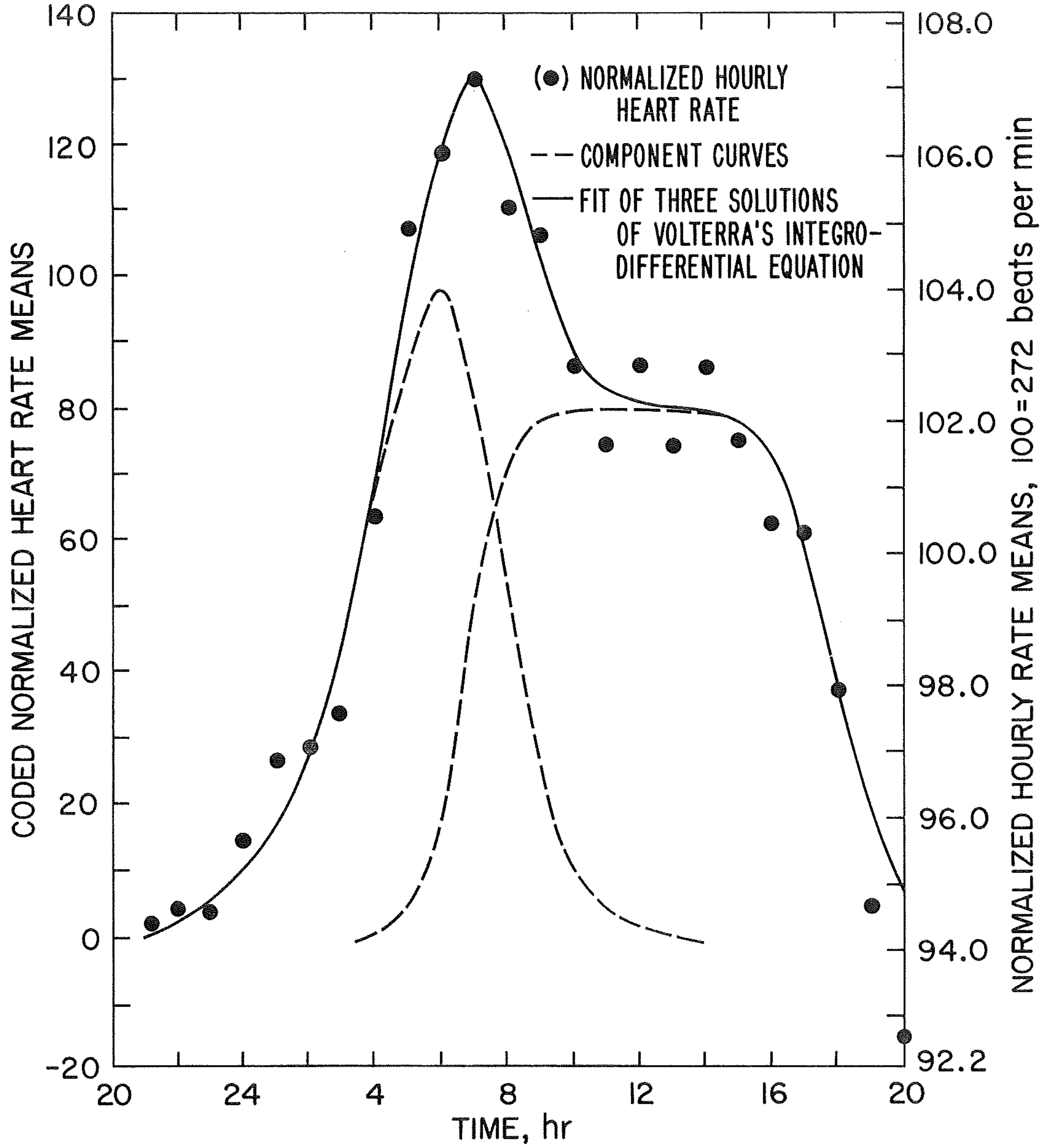


Figure 8.

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